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Human Genome Epidemiology (HuGE) Review

Association between the Transforming Growth Factor Alpha Gene and Nonsyndromic Oral Clefts: A HuGE Review

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Transforming growth factor alpha (TGFA) is a well-characterized mammalian growth factor. Since the first report of an association between DNA sequence variants at the *TGFA* genetic locus and nonsyndromic oral clefts, 47 studies have been carried out, producing conflicting results. In this review, the author synthesizes findings from published reports on the association between the *TGFA* gene and clefting in humans. Bias, lack of statistical power, and genuine population diversity can explain the diverse results. In the aggregate, *TGFA* is probably a genetic modifier of clefting in humans, which is consistent with the oligogenic model suggested for nonsyndromic oral clefts.

cleft lip; cleft palate; epidemiology; genetics; *TGFA*; transforming growth factor alpha

Abbreviations: CI, confidence interval; FGFR1, fibroblast growth factor receptor 1; IRF6, interferon regulatory factor 6; LOD, logarithm of the odds; MSX1, muscle segment homeobox 1; MTHFR, 5,10-methylenetetrahydrofolate reductase; PAX9, paired box 9; PCR, polymerase chain reaction; TGFA, transforming growth factor alpha; TGFB3, transforming growth factor beta 3.

GENE

Transforming growth factor alpha (TGFA) is a well-characterized mammalian growth factor. It has been mapped to chromosome 2p13 (1, 2), comprises 80 kilobases of genomic DNA, and consists of six exons (sizes: exon 1, 40 base pairs; exon 2, 57 base pairs; exon 3, 118 base pairs; exon 4, 150 base pairs; exon 5, 110 base pairs) (figure 1).

Expression of the *TGFA* gene occurs in a wide spectrum of normal tissue from the preimplantation period in mouse embryos to adult life (3–8). During craniofacial development, *TGFA* is expressed at the medial edge epithelium of fusing palatal shelves (9, 10). In palatal cultures, *TGFA* promotes synthesis of extracellular matrix and mesenchymal cell migration, thereby ensuring the strength of the fused palate (11).

Although *Tgfa* is expressed in mice during palatogenesis, mice with a null mutation of the *Tgfa* gene have abnormal skin, hair, and eyes but do not have oral clefts (12, 13). Newborn epidermal growth factor receptor-negative/-negative mice have a high incidence of cleft palate, and this may explain the genetic correlation of human oral clefts with polymorphisms in *TGFA* (14), given that TGFA is a likely ligand for epidermal growth factor receptor. Cleft lip with or without cleft palate was first associated with polymorphisms in *TGFA* in 1989 (15), and the topic was reviewed in 1997 (16), 2001 (17, 18), and 2002 (19–21).

GENE VARIANTS

An extensive Human Genome Epidemiology (HuGE) review of the gene variants for *TGFA* was performed. Medline, PubMed, and EMBASE were searched using the keywords ''transforming growth factor alpha'' and ''TGFA.'' Additional search words included ''oral clefts,'' ''cleft lip and palate,'' and ''orofacial clefts.'' Reference lists from published articles were also reviewed, and journals related

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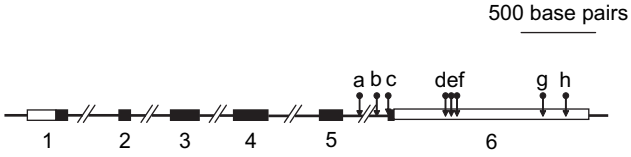

FIGURE 1. Genomic structure of transforming growth factor alpha (*TGFA*). Based on the paper by Vieira et al. (114), with corresponding GeneBank entry AH013033. Black boxes are coding regions; white boxes are untranslated regions. Numbers indicate exons. Arrows and letters indicate the locations of the most-studied *TGFA* variants: (*a*) *Taq*I; (*b*) *Rsa*I; (*c*) C3296T (C-to-T substitution at nucleotide 3296); (*d*, *e*, *f*) primer K; (*f*) C3827T (C-to-T substitution at nucleotide 3827); (*g*) primer P; and (*h*) *Bam*HI.

specifically to birth defects and clefting were searched by hand. The review included papers written in English, French, Spanish, and Portuguese, as well as Chinese or Japanese papers with English abstracts, that were published between 1986 and 2005.

Currently, 356 single nucleotide polymorphisms and 20 insertion/deletion polymorphisms can be found in the May 2004 human genome assembly freeze in the University of California, Santa Cruz genome browser (http://www.genome.ucsc.edu/). Table 1 and figure 1 present the variants found to be most studied for oral clefts. There is a lack of information regarding the potential function of the variants presented in table 1. The only consideration of this issue was in an Iowa study of cases of cleft palate only and the

TABLE 1. Variants of the transforming growth factor alpha (*TGFA*) gene

Variant	Location	Allele	Reference no.
Marker 1	Insertion of C, 53 base pairs from donor site of exon 1	Wild type: TCCCCGCACCGCGGCGCC	26
		Rare: - - - - CACCGCGGCGCC	
Marker 3	G→A, 23 base pairs from donor site of exon 3	Wild type: TTCTCCTGGAGATCTGGG	26
		Rare: - - - - A - - - - -	
*Rsa*I	Intron 5, 177 base pairs upstream of the acceptor site of exon 6	B1 (wild type): ACTGAAAGTATTATGTCA	25
		B2 (rare): - - - - - - C - G - -	
*Taq*I	Intron 5, 1,602 base pairs upstream of the acceptor site of exon 6	C1 (wild type): AGGTCTCTAATGACCTTA	24
		C2 (rare): - - - - - - - -	
Marker 6A	Exon 6, one base pair from stop codon, C3296T (Val→Val)	Wild type: TGGTCTGAAGAGCCCAGA	26
		Rare: - - T - - - - - - -	
*Hin*fI	3′-UTR*, C3803T	Allele 1 (wild type): AACCACAAGACCCTCAAC	25
		Allele 2 (rare): - - - - - - T - - - -	
Primer K	3′-UTR, three SNPs*: G3798A, C3803T, C3827T	Allele 1: - A - C - T -	22
		Allele 2: - G - C - T -	
		Allele 3 (wild type): - G - C - C -	
		Allele 4: - G - T - T -	
Marker 2A	3′-UTR, four SNPs: G3822A, C3827T, T3851C, A3879G	Wild type: - G - C - T - A -	26
		Rare 1: - A - T - T - A -	
		Rare 2: - G - T - T - A -	
		Rare 3: - G - T - C - A -	
		Rare 4: - G - C - T - G -	
Marker 2	3′-UTR, deletion of A, position 3961	Wild type: ATGTAAAAAGTATAAAAC	26
		Rare: ATGTAAAA-GTATAAAAC	
Marker H3	3′-UTR, A4237G	Wild type: TCTGTTGGGGAGAGAGGA	26
		Rare: - - - - - - G - - - -	
Marker H4	3′-UTR, G4329A	Wild type: GTGAGCCCTCGGTAAGTA	26
		Rare: - - - - - A - - - -	
Marker H6	3′-UTR, deletion of T, position 4520	Wild type: TAATTTTTTTTTCCTCAT	26
		Rare: TAATTTTTTTT CCTCAT	
Primer P	3′-UTR, four-base-pair deletion, position 4932	Wild type: TTTCTCTTTATTTTTTTT	22
		Rare: TTTCT - - ATTTTTTTT	
*Bam*HI	3′-UTR, C5560A	A1 (wild type): AGCATTGGCTCCCTCTGC	25
		A2 (rare): - - - - A - - - - -	

* UTR, untranslated region; SNP, single nucleotide polymorphism.

TABLE 2. Distribution of transforming growth factor alpha (*TGFA*) *Bam*HI alleles*

Location of study	Ethnicity	No. of subjects	Source of subjects	Genotype			A1A1 frequency (%)	95% confidence interval	Year	Reference no.
				A1A1	A1A2	A2A2				
Australia	European descent	112†	Unspecified	0	26	82	0	0, 1.6	1992	28
Chile	Chilean	100	Blood donors	5	12	83	5.0‡	4.2, 5.8	1995	98
England	European descent	60	Relatives of persons affected by cystic fibrosis and research colleagues	0	16	44	0	0, 18.0	1992	36
France	Alsatian descent	99	Birth registry	0	15	84	0	0.0, 0.9	1992	29
	European descent and African descent	10	Cancer cell lines	1	4	5	10.0	6.0, 14.0	1993	29
United States, Iowa	European descent	96	Hospital	1	23	72	1.0	0.01, 15.9	1989	15

* Reference 125 was not included because studied samples were relatives of persons affected by clefting.
† Four persons were reported to have a third allele; two were A1A3 and two were A2A3.
‡ Not in Hardy-Weinberg equilibrium.

primer K variant (22). The authors located this variant in the 3′-untranslated region of the gene (figure 1), within the same region as a transcribed 350-nucleotide polyadenylated, antisense mRNA species (23). If the antisense mRNA regulates the expression of *TGFA* by interacting with the K region of *TGFA*, the primer K variant may contribute to the cleft of the palate.

The clinical studies reviewed were either case-control or family-based in design. The *Taq*I variant, first reported in 1987 (24) and characterized as a four-base-pair deletion in intron 5, is the one most studied in case-control studies of cleft lip and palate. In addition, the *Bam*HI and *Rsa*I variants (25) and the single nucleotide polymorphisms C3296T (rs2166975; a C-to-T substitution at nucleotide 3296) and C3827T (rs1058213; a C-to-T substitution at nucleotide 3827) (26) have also been studied. The published genotype frequencies for the *Bam*HI variant are shown in table 2, and those for the *Rsa*I variant are shown in table 3. Table 4 presents genotype frequencies for the *Taq*I variant from published studies and includes the frequencies of the populations in the Human Genome Diversity Cell Line Panel (27). The Diversity Cell Line Panel is a resource of 1,064 cultured lymphoblastic cell lines obtained from persons in different world populations. Lymphoblastic cell lines were collected from various laboratories by the Human Genome Diversity Project and the Fondation Jean Dausset–CEPH [Centre d'Etude du Polymorphisme Humain] to obtain unlimited supplies of DNA for studies of sequence diversity and history in modern human populations. Each cell line comes from a single individual. Samples were originally collected under nonrandom selection. The panel contains lymphoblastic cell lines from human populations on all

TABLE 3. Distribution of transforming growth factor alpha (*TGFA*) *Rsa*I alleles*

Location of study	Ethnicity	No. of subjects	Source of subjects	Genotype			B1B1 frequency (%)	95% confidence interval	Year	Reference no.
				B1B1	B1B2	B2B2				
England	European descent	59	Relatives of persons affected by cystic fibrosis and research colleagues	10	22	27	17.0†	16.6, 17.4	1992	36
France	Alsatian descent	99	Birth registry	11	36	52	11.0	7.6, 14.4	1992	29
	European descent and African descent	10	Cancer cell lines	2	3	5	20.0†	17.0, 23.0	1993	29
United States										
Iowa	European descent	101	Hospital	11	32	58	11.0†	10.7, 11.2	1989	15
Philadelphia, Pennsylvania	African-American	8	Hospital	0	4	4	0	0, 0.5	1993	37
	Asian-American	6		0	2	4	0	0, 0.25		
	European descent	84		8	28	48	10.0†	9.2, 10.8		

* Reference 125 was not included because studied samples were relatives of persons affected by clefting.
† Not in Hardy-Weinberg equilibrium.

TABLE 4. Worldwide distribution of transforming growth factor alpha (*TGFA*) *Taq*I alleles*

Location of study	Ethnicity	No. of subjects	Source of subjects	Genotype			C2C2 frequency (%)	95% confidence interval	Year	Reference no.
				C1C1	C1C2	C2C2				
Africa										
Algeria	Mozabite	30	CEPH†	30	0	0	0			JCM†
Central African Republic	Biaka Pygmies	36	CEPH	18	12	6	16.7	0.0, 49.7		JCM
Congo	Mbuti Pygmies	15	CEPH	11	3	1	6.7	0.0, 19.7		JCM
Kenya	Bantu	12	CEPH	11	1	0	0	0.0, 4.5		JCM
Namibia	San	8	CEPH	4	3	1	12.5	0.0, 51.3		JCM
Nigeria	Yoruba	25	CEPH	21	4	0	0	0.0, 9.0		JCM
Senegal	Mandenka	24	CEPH	19	5	0	0	0.0, 13.0		JCM
South Africa	Bantu	8	CEPH	8	0	0	0			JCM
Asia/Middle East										
Cambodia	Cambodian	11	CEPH	11	0	0	0			JCM
China, Guangdong	Han	136	Hospital	114	21	1	0.7	0.0, 2.7	2004	99
China	Han	45	CEPH	30	15	0	0	0.0, 25.0		JCM
	Tujia	10	CEPH	10	0	0	0			JCM
	Yizu	10	CEPH	10	0	0	0			JCM
	Miaozu	10	CEPH	8	2	0	0	0.0, 12.5		JCM
	Oroqen	10	CEPH	7	2	1	10.0	0.0, 34.3		JCM
	Daur	10	CEPH	8	2	0	0	0.0, 12.5		JCM
	Mongola	10	CEPH	10	0	0	0			JCM
	Hezchen	10	CEPH	8	1	1	10.0	0.0, 6.25		JCM
	Xibo	9	CEPH	9	0	0	0			JCM
	Uygur	10	CEPH	10	0	0	0			JCM
	Dai	10	CEPH	6	4	0	0	0.0, 33.3		JCM
	Lahu	10	CEPH	9	1	0	0	0.0, 5.5		JCM
	She	10	CEPH	7	3	0	0	0.0, 21.4		JCM
	Naxi	10	CEPH	9	1	0	0	0.0, 5.5		JCM
	Tu	10	CEPH	7	3	0	0	0.0, 21.4		JCM
Israel	Bedouin	49	CEPH	46	3	0	0	0.0, 3.0		JCM
	Druze	48	CEPH	48	0	0	0			JCM
	Palestinian	51	CEPH	47	4	0	0	0.0, 4.0		JCM
Japan	Japanese	117	Unspecified	91	26	0	0	0.0, 14.0	1996	100
	Japanese	31	CEPH	23	7	1	4.0	0.0, 15.2		JCM
	Japanese	312	Hospital	240	59	13	4.2‡	4.0, 4.4	1997	101
Pakistan	Brahui	25	CEPH	25	0	0	0			JCM
	Balochi	25	CEPH	25	0	0	0			JCM
	Hazara	25	CEPH	23	1	1	4.0‡	1.8, 6.2		JCM
	Makrani	25	CEPH	24	1	0	0	0.0, 2.0		JCM
	Sindhi	25	CEPH	25	0	0	0			JCM
	Pathan	25	CEPH	24	1	0	0	0.0, 2.0		JCM
	Kalash	25	CEPH	24	1	0	0	0.0, 2.0		JCM
	Burusho	25	CEPH	23	1	1	4.0‡	1.8, 6.2		JCM
Philippines	Filipino	792	Hospital	674	116	2	2.0	0.0, 10.6	1997	102
Russia, Caucasus	Adygei	17	CEPH	14	3	0	0	0.0, 10.7		JCM
Russia, Siberia	Yakut	25	CEPH	16	8	1	4.0	0.0, 25.0		JCM
Vietnam	Vietnamese	10	Hospital	10	0	0	0		1997	101

Table continues

TABLE 4. Continued

Location of study	Ethnicity	No. of subjects	Source of subjects	Genotype			C2C2 frequency (%)	95% confidence interval	Year	Reference no.
				C1C1	C1C2	C2C2				
Europe										
Denmark	European descent	457	Birth registry	344	102	11	2.4	0.0, 17.2	1999	41
England	European descent	60	Relatives of persons affected by cystic fibrosis and research colleagues	55	5	0	0	0.0, 4.5	1992	36
France	French	29	CEPH	25	3	1	3.4	0.0, 9.4		JCM
	French Basque	24	CEPH	18	6	0	0	0.0, 16.7		JCM
Italy	North Italian	14	CEPH	11	3	0	0	0.0, 13.6		JCM
	Sardinian	28	CEPH	26	2	0	0	0.0, 3.8		JCM
	Tuscan	8	CEPH	7	1	0	0	0.0, 7.1		JCM
Norway	Norwegian	262	Hospital	193	62	7	2.7	2.5, 2.9	2003	75
Russia	Russian	25	CEPH	22	3	0	0	0.0, 6.8		JCM
Scotland, Orkney Islands	Orcadian	16	CEPH	11	5	0	0	0.0, 22.7		JCM
North America										
Mexico	Pima	25	CEPH	25	0	0	0			JCM
	Maya	25	CEPH	25	0	0	0			JCM
United States										
(Location not reported)	Amerindian	4	CEPH	4	0	0	0			JCM
Iowa	European descent	98	Hospital	89	8	1	1.0	0.0, 5.4	1989	15
Philadelphia, Pennsylvania	African-American	8	Hospital	5	3	0	0	0.0, 30.0	1993	37
	Asian-American	6		4	2	0	0	0.0, 25.0		
	European descent	84		70	13	1	1.2	0.0, 10.4		
Maryland	European descent	284	Birth registry	239	44	1	0.4	0.0, 9.4	1995	39
California	European descent	379	Birth registry	321	55	3	0.8	0.0, 9.3	1996	40
	Hispanic	175		164	9	2	1.1	0.0, 3.8		
Maryland	African-American	87	Hospital	79	8	0	0	0.0, 4.4	1997	103
	European descent	45		43	2	0	0	0.0, 2.3		
Puerto Rico	Puerto Rican	132	Population	107	25	0	0		2005	70
Oceania										
Australia	European descent	63	Unspecified	58	4	1	1.6	0.0, 5.0	1988	67
Australia	European descent	100	Unspecified	90	9	1	1.0	0.0, 6.0	1991	104
Papua New Guinea, Bougainville	Melanesian	22	CEPH	22	0	0	0			JCM
Papua New Guinea	Papuan	17	CEPH	17	0	0	0			JCM
South America										
Brazil, Rio de Janeiro	Brazilian	199	Hospital	184	14	1	0.5	0.2, 1.0	2004	59
Brazil, São Paulo	Brazilian	214	Hospital	185	29	0	0	0.0, 8.0	2004	105
Brazil, Ceará	Brazilian	171	Hospital	159	12	0	0	0.0, 3.7	2004	105
Brazil	Karatiana	24	CEPH	24	0	0	0			JCM
Brazil	Surui	21	CEPH	21	0	0	0			JCM
Chile	Chilean	51	Blood donors	44	6	1	2.0	0.0, 8.8	1995	106
Colombia	Colombian	13	CEPH	13	0	0	0			JCM

* References 35, 46, and 125 were not included because studied samples were relatives of persons affected by clefting.
† CEPH, Centre d'Etude du Polymorphisme Humain; JCM, Jeffrey C. Murray (University of Iowa, personal communication, 2003).
‡ Not in Hardy-Weinberg equilibrium.

TABLE 5. Worldwide distribution of the transforming growth factor alpha (*TGFA*) C3296T (C-to-T substitution at nucleotide 3296) and C3827T (C-to-T substitution at nucleotide 3827) alleles*

Location of study	Ethnicity	No. of subjects	Source of subjects	C3296T genotype			TT frequency (%)	95% CI†	C3827T genotype			TT frequency (%)	95% CI
				CC	CT	TT			CC	CT	TT		
Africa													
Algeria	Mozabite	30	CEPH†,‡	20	9	1	3.3	0.0, 22.5	25	4	1	3.3	0.0, 8.0
Central Africa Republic	Biaka Pygmies	36	CEPH	34	2	0	0	0.0, 2.9	36	0	0	0	
Congo	Mbuti Pygmies	15	CEPH	15	0	0	0		15	0	0	0	
Kenya	Bantu	12	CEPH	12	0	0	0		12	0	0	0	
Namibia	San	7	CEPH	7	0	0	0		7	0	0	0	
Nigeria	Yoruba	25	CEPH	19	5	1	4.0	0.0, 13.1	23	2	0	0	0.0, 4.3
Senegal	Mandenka	24	CEPH	20	4	0	0	0.0, 10.0	24	0	0	0	
South Africa	Bantu	8	CEPH	7	1	0	0	0.0, 7.1	7	1	0	0	0.0, 7.1
Asia/Middle East													
Cambodia	Cambodian	11	CEPH	11	0	0	0		8	3	0	0	0.0, 18.7
China	Han	45	CEPH	36	6	3	6.7	0.0, 15.0	36	5	4	8.9§	2.0, 15.8
	Tujia	10		8	2	0	0	0.0, 12.5	6	3	1	10.0	0.0, 25.0
	Yizu	10		10	0	0	0		10	0	0	0	
	Miaozu	10		8	2	0	0	0.0, 12.5	7	1	2	20.0§	12.9, 27.1
	Oroqen	10		10	0	0	0		10	0	0	0	
	Daur	10		8	2	0	0	0.0, 12.5	8	1	1	10.0§	3.8, 16.2
	Mongola	10		9	1	0	0	0.0, 5.5	7	2	1	10.0	0.0, 24.2
	Hezchen	10		10	0	0	0		10	0	0	0	
	Xibo	9		7	2	0	0	0.0, 14.2	5	3	1	11.1	0.0, 41.1
	Uygur	10		8	2	0	0	0.0, 12.5	7	1	2	20.0	12.9, 27.1
	Dai	10		9	1	0	0	0.0, 5.5	9	1	0	0	0.0, 5.5
	Lahu	10		9	1	0	0	0.0, 5.5	6	3	1	10.0	0.0, 35.0
	She	10		10	0	0	0		10	0	0	0	
	Naxi	10		8	2	0	0	0.0, 12.5	6	2	2	20.0§	3.3, 36.7
	Tu	10		9	1	0	0	0.0, 5.5	9	0	1	10.0	0.0, 20.0
Israel	Bedouin	49	CEPH	45	4	0	0	0.0, 4.4	47	1	1	2.0§	1.0, 3.0
	Druze	48		48	0	0	0		47	1	0	0	0.0, 1.0
	Palestinian	51		49	2	0	0	0.0, 2.0	48	1	2	3.9§	2.9, 4.9
Japan	Japanese	31	CEPH	26	5	0	0	0.0, 9.6	25	1	5	16.1§	14.1, 18.1

Table continues

continents. The maximum number of lymphoblastic cell lines from a population is 51. Twenty-five to 49 lymphoblastic cell lines are available from each of 21 population samples. Fourteen Chinese minority groups are represented by only 9–10 lymphoblastic cell lines each.

Table 5 presents the genotype frequencies of the C3296T and C3827T variants.

Most of the studies from which data were abstracted and are presented in tables 2–5 were not population-based, with the exception of studies from Denmark, France, Norway, and the US states of California and Maryland. The Filipino data shown in table 4 were from a hospital-based study. The frequencies presented in tables 2–5 come from controls/unaffected persons.

Most of the studies described in tables 2–5, as well as most of the populations from the Diversity Cell Line Panel, were small, as evidenced by wide 95 percent confidence intervals for the frequency of the least common genotype. In many instances in tables 2–5, the data do not suggest Hardy-Weinberg equilibrium. The studies in Hardy-Weinberg disequilibrium either used a convenient sample as controls or obtained data from a very small sample. In tables 4 and 5, many populations from the Diversity Cell Line Panel were in Hardy-Weinberg disequilibrium, probably because sample sizes were very small. Genotyping error also cannot be discounted.

For tables 2–5, in the case of overlap between studies, the report with the most thorough description was used to

TABLE 5. Continued

Location of study	Ethnicity	No. of subjects	Source of subjects	C3296T genotype			TT frequency (%)	95% CI	C3827T genotype			TT frequency (%)	95% CI
				CC	CT	TT			CC	CT	TT		
Pakistan	Brahui	25	CEPH	24	1	0	0	0.0, 2.0	21	4	0	0	0.0, 9.5
	Balochi	25		24	1	0	0	0.0, 2.0	23	1	1	4.0§	1.9, 6.1
	Hazara	25		21	4	0	0	0.0, 9.5	17	5	3	12.0	0.0, 36.7
	Makrani	25		25	0	0	0		23	2	0	0	0.0, 4.3
	Sindhi	25		23	2	0	0	0.0, 4.3	22	1	2	8.0§	5.8, 10.2
	Pathan	25		23	2	0	0	0.0, 4.3	21	3	1	4.0	0.0, 11.1
	Kalash	25		24	1	0	0	0.0, 2.0	25	0	0	0	
	Burusho	25		22	2	1	4.0	0.0, 8.5	24	0	1	4.0	0.0, 24.0
Russia, Caucasus	Adygei	17	CEPH	15	2	0	0	0.0, 6.7	17	0	0	0	
Russia, Siberia	Yakut	22	CEPH	3	0	0	0	0.0, 6.8	19	4	2	8.0	0.0, 18.5
Europe													
France	French	29	CEPH	29	0	0	0		26	2	1	3.4	0.0, 7.2
	French Basque	24		23	1	0	0	0.0, 2.1	21	1	2	8.3§	6. 0, 10.6
Italy	North Italian	14	CEPH	12	2	0	0	0.0, 8.3	13	1	0	0	0.0, 3.8
	Sardinian	28		25	3	0	0	0.0, 6.0	27	0	1	3.8	0.0, 27.0
Russia	Russian	25	CEPH	22	3	0	0	0.0, 6.8	21	2	2	8.0§	3.3, 12.7
Scotland, Orkney Islands	Orcadian	16	CEPH	14	2	0	0	0.0, 7.1	15	1	0	0	0.0, 3.3
North America													
Mexico	Pima	25	CEPH	24	0	1	4.0	0.0, 24.0	24	1	0	0	0.0, 2.0
	Maya	25		23	1	1	4.0§	1.9, 6.1	23	1	1	4.0§	1.9, 6.1
United States	Amerindian	4	CEPH	4	0	0	0		4	0	0	0	
Oceania													
Papua New Guinea, Bougainville	Melanesian	22	CEPH	6	15	1	4.0	0.0, 84.0	5	1	16	72.7§	62.7, 82.7
Papua New Guinea	Papuan	17	CEPH	3	13	1	4.0	0.0, 46.1	3	2	12	70.5	41.1, 99.9
South America													
Brazil, Rio de Janeiro	Brazilian	204–207	Hospital (Vieira et al. (59))	162	44	1	0.5	0.36, 0.64	176	21	7	3.4	2.9, 4.1
Brazil	Karatiana	24	CEPH	23	0	1	4.2	0.0, 27.2	24	0	0	0	
Brazil	Surui	21	CEPH	21	0	0	0		21	0	0	0	
Colombia	Colombian	13	CEPH	10	3	0	0	0.0, 15.0	10	3	0	0	0.0, 15.0

* References 35 and 114 were not included because studied samples were relatives of persons affected by clefting.
† CI, confidence interval; CEPH, Centre d'Etude du Polymorphisme Humain.
‡ All CEPH data were provided by Dr. Jeffrey C. Murray (University of Iowa, personal communication, 2003).
§ Not in Hardy-Weinberg equilibrium.

abstract genotype frequency data. The few inconsistencies between data presented in tables 2–5 and data presented in later tables are due to small differences between the reported total numbers and the actual genotype information available.

There is a wide range in the *TGFA Taq*I, C3296T, and C3827T (tables 4 and 5) allele frequencies across different studies. Some populations show a remarkably high frequency of the *TGFA Taq*I C2 (rare) allele, including Biaka Pygmies, Chinese Han, Danish, Japanese, and Filipinos. For the C3296T and C3827T alleles, the Melanesians and Papuans have the T allele for both loci as the most common one. The C allele is the most common for both variants in all other populations studied.

As ancestral haplotypes propagate through a population, their physical length is reduced by recombination events. Thus, genotypes at nearby markers are not independent, and their association may reflect ancestral founding haplotypes. Most of the *TGFA*-cleft association studies relate to *Taq*I, *Bam*HI, and *Rsa*I polymorphisms, but there is no information on linkage disequilibrium for these three markers. Therefore, linkage disequilibrium between *Taq*I and *Bam*HI,

TABLE 6. Results of linkage disequilibrium analysis for the transforming growth factor alpha (*TGFA*) variant alleles *Taq*I, *Bam*HI, and *Rsa*I

Study location and population	Reference no.	*Taq*I–*Bam*HI			*Taq*I–*Rsa*I			*Bam*HI–*Rsa*I		
		χ^2 value	*p* value	No. of chromosomes	χ^2 value	*p* value	No. of chromosomes	χ^2 value	*p* value	No. of chromosomes
Australia, European descent	28	0.846	0.3577	163						
France	29							14.166	0.0001	176
United States, Iowa, European descent	15	1.032	0.3097	161	3.103	0.0781	161	31.572	<0.00001	161

*Taq*I and *Rsa*I, and *Bam*HI and *Rsa*I marker alleles was calculated from published haplotype data (15, 28, 29) (table 6). *Taq*I and *Bam*HI marker alleles are not in linkage disequilibrium. However, *Taq*I and *Rsa*I marker alleles present borderline linkage disequilibrium, while *Bam*HI and *Rsa*I are strongly linked. Future studies should avoid generating data for both of the strongly linked variants. Combined genotypes of the *Taq*I and *Bam*HI variants would provide the most informative data.

Linkage disequilibrium analysis of the three variants is also reported for the Human Genome Diversity Cell Line Panel (table 7). For this analysis, the populations were pooled by geographic origin, and the D' statistic was calculated using the software GOLD (30). This statistic measures the difference between the observed and expected (under independence) numbers of haplotypes bearing one marker allele and the other marker allele. D' depends strongly on marker allele and disease allele frequencies. Values higher than 0.9 are considered to be in strong linkage disequilibrium, and a value equal to 1.0 indicates complete linkage disequilibrium (31).

Linkage disequilibrium calculations can provide evidence for how close in time mutation events resulting in single nucleotide polymorphisms occurred in a given population. The *TGFA Taq*I allele is in weak linkage disequilibrium with the C3296T allele in Adygei and Russians ($D' = 0.071$)

TABLE 7. Results of linkage disequilibrium analysis for transforming growth factor alpha (*TGFA*) variant alleles in the Human Genome Diversity Cell Line Panel*

Study population	D' value					
	*Taq*I–C3296T†	No. of subjects	*Taq*I–C3827T†	No. of subjects	C3296T–C3827T	No. of subjects
Adygei + Russian	0.071‡	42	1.0	42	0.138‡	42
African§	1.0	126	1.0	125	1.0	125
Algerian	0.0¶	30	0.0¶	30	0.515	30
Brazilian Indian (Karatiana + Surui)	0.0¶	45	0.0¶	45	0.0¶	45
Cambodian + Oceanian (Melanesian + Papuan)	0.0¶	50	0.0¶	50	0.660	50
Chinese Han	1.0	44	1.0	44	0.513	45
Chinese (minorities)#	1.0	139	1.0	139	0.852	139
Colombian	0.0¶	13	0.0¶	13	0.604	13
French + French Basque	1.0	53	0.008‡	53	1.0	53
Israelis (Bedouin + Druze + Palestinian)	1.0	144	1.0	147	0.484	145
Italian (North Italian + Sardinian + Tuscan)	1.0	50	1.0	50	0.638	50
Japanese	1.0	31	1.0	31	1.0	31
Mexican Indian (Pima + Maya)	0.0¶	47	0.0¶	50	0.472	47
Orcadian (Scotland, Orkney Islands)	1.0	16	1.0	16	1.0	16
Pakistani**	0.077‡	203	1.0	203	0.636	204
Yakut (Siberia)	1.0	24	1.0	24	1.0	25

* Frequency data were provided by Dr. Jeffrey C. Murray (University of Iowa, personal communication, 2003).
† C3296T is a C-to-T substitution at nucleotide 3296; C3827T is a C-to-T substitution at nucleotide 3827.
‡ Based on fewer than five heterozygotes.
§ Combined samples from the Central African Republic, Congo, Senegal, Nigeria, Kenya, Namibia, and South Africa.
¶ Uninformative.
# Combined samples of Tujia, Yizu, Miaozu, Oroqen, Daur, Mongola, Hezhen, Xibo, Uygur, Dai, Lahu, She, Naxi, and Tu.
** Combined samples of Brahui, Balochi, Hazara, Makrani, Sindhi, Pathan, Kalash, and Burusho.

and in Pakistanis ($D' = 0.077$), suggesting that these two mutation events are ancient (probably more than 50–100 generations old) or arose independently two or more times. The *TGFA Taq*I site shows weak linkage disequilibrium with the C3827T allele in French and French Basques (0.008). Several population groups have the C3296T and C3827T alleles in weak linkage disequilibrium.

DISEASES AND THEIR ASSOCIATIONS

Nonsyndromic oral clefts

Isolated or nonsyndromic oral clefts (those occurring in people with no other structural or developmental abnormalities) are common congenital anomalies in humans. Typically, oral clefts are anatomically divided into two groups: cleft lip with or without cleft palate (hereafter called cleft lip/palate) and cleft palate only. The prevalence at birth of cleft lip/palate among persons of European ancestry is generally near 1 in 1,000 livebirths; for cleft palate only, the prevalence at birth is lower (1 in 2,500 livebirths), but there is substantial variability and higher prevalence at birth in Northern Europeans. The only demographic variable that has been consistently associated with the prevalence of nonsyndromic oral clefts is ethnicity. Compared with European descendants, prevalence is higher in Asians and American Indians and lower in persons of African descent (32, 33).

Since the first report of an association between *TGFA* and oral clefts (15), some studies, but not all, have replicated this finding. Tables 8–10 summarize results from studies that investigated the possible association/linkage between oral clefts and the *TGFA* locus. The tables present data from all reports available, including multiple reports on basically the same data set, to allow appreciation of the different findings obtained within the same population.

The first studies suggested a stronger genetic effect than was found by subsequent studies. Both bias and genuine population diversity might explain why early studies tended to overestimate the disease predisposition conferred by *TGFA* polymorphisms (17). Tables 2–5 show clearly that most of the studies contained very small series. Any future publication of the results of an association study (whether negative or positive) should be accompanied by a meta-analysis of all similar studies. Accordingly, individual researchers should also publish or make easily available information that will facilitate future meta-analysis, including relevant genotype and phenotype data (20).

There has been considerable variation in study designs, markers used, and percentages of patients with a positive family history, such that direct comparisons are difficult. The wide range in *TGFA Taq*I allele frequencies across different studies (3–20 percent) suggests that heterogeneity between populations may exist (34, 35). A meta-analysis (16) showed evidence of statistically significant heterogeneity between European-descendant cleft lip/palate patients from different studies before 1997, which could reflect differences in allele frequency, percentage of positive family history, cleft severity, and ethnicity. Interestingly, this same study reported similar allele frequencies for controls comprising Australians of predominantly Anglo-Celtic descent, French of Alsatian ancestry, Britons, and US European descendants from California, Iowa, Maryland, or Philadelphia, Pennsylvania (15, 28, 36–40). However, the present review does not support this statement (table 4). Much of the variation we can see in the *Taq*I, C3296T, and C3827T marker allele frequencies could be due to chance, since many of the series were small. In addition, there is evidence of selection bias for these non-population-based series.

The author of the meta-analysis (16) concluded that the lack of significant heterogeneity between such diverse groups of European descendants suggests that *TGFA* allele frequencies are unlikely to be dramatically influenced by ethnicity. However, this may be not true for all cases. Danes have a frequency of the ''rare'' *TGFA Taq*I allele that is at least 10 percent higher than that of other European populations tested. In addition, the frequency of cleft lip/palate in Scandinavia is among the highest in the world. When case-control studies are conducted in regions admixed by Danish migrants, investigators may inadvertently select a population that has a higher frequency of the ''rare'' *TGFA* marker allele in the case group (41). This could explain the results of studies conducted in the US state of Iowa (15, 22, 26, 34, 42–44), where there is substantial Northern European mixing (45).

However, it is unlikely that the association between *TGFA* and oral clefts that has been reported in studies using family-based controls or the transmission disequilibrium test is due to the confounding influence of ethnicity (i.e., population stratification). These findings provide evidence against the ethnicity bias (34, 46–48), because the affected-family-based controls and transmission disequilibrium tests are not subject to the potentially confounding influence of population stratification (49).

There is a consistent pattern of positive findings in Australia, Chile, France, and Great Britain and negative findings in some Asian populations, such as Asian Indians, Chinese, Filipinos, and Turks (tables 8 and 10). Studies in North American populations present somewhat contradictory findings (see table 8), which may result from a lack of statistical power. If *TGFA* has a small effect on clefting, this may be missed in assessments of both type I error and type II error.

The evidence regarding an association between genetic variation at the *TGFA* locus and cleft lip/palate was considered inconclusive in the first meta-analysis (16). A second meta-analysis showed a small effect of the *TGFA Taq*I marker (17). The current review revisited additional studies that included not only case-control and family-based approaches but also linkage. There is evidence that *TGFA* plays a small but significant role in cleft lip/palate and that lack of power to detect very mild effects is the main reason for the conflicting results. Investigators should move forward in the direction of functional studies to define the role of the *TGFA* variants described in table 1.

The first genome-wide scan published for cleft lip/palate in European descendants studied 92 British affected sibling pairs and found maximum logarithm of the odds (LOD) scores equal to 0.66 at chromosome 2p13 (the *TGFA* locus) (50). This study also was unable to demonstrate the involvement of a single locus of major effect in cleft lip/palate. Genome-wide scans done in Chinese, Syrian, Turkish, and West Bengali families also found positive LOD scores for

TABLE 8. Results from case-control studies of the association between the transforming growth factor alpha (*TGFA*) gene and oral clefts*

Location of study	Reference no.	*TGFA* genotype	Sample size and type		Reported results for association†	Highlights
			Cases	Controls (source)		
Australia	104	*Taq*I	96 CL/P‡	100 (unspecified)	Association; $p = 0.0003$ (two-tailed exact test)	63 controls were taken from the paper by Hayward et al. (24).
	28	*Taq*I and *Bam*HI	117 CL/P	113 (33 geriatric patients, 34 laboratory workers, 27 spouses of patients with inherited disorders, and 19 mothers of twins)	Borderline association; $p = 0.049$ for *Taq*I and $p = 0.053$ for *Bam*HI (two-tailed exact test)	There is overlap with the cases in the paper by Chenevix-Trench et al. (104), but a different set of controls was used. 59% of cases had a positive family history of clefts.
Chile	98	*Bam*HI	21 CL/P	16 (blood donors)	No association	There is overlap of the samples in these three reports, but the association grows stronger as the sample size grows bigger.
	106	*Taq*I and *Bam*HI	39 CL/P	51 (blood donors)	Association; $p = 0.0143$ for *Bam*HI (two-tailed exact test)	
	107	*Bam*HI	65 CL/P	100 (blood donors)	Association; $p = 0.004$ (two-tailed exact test)	
Denmark	41	*Taq*I	233 CL/P; 83 CPO‡	604 (birth registry)	No association	
England	36	*Taq*I, *Bam*HI, *Rsa*I	57 CL/P	60 (relatives of persons affected by cystic fibrosis and research colleagues)	Association; $p < 0.001$ for *Taq*I (two-tailed exact test)	37% of cases had a positive family history of clefts.
France	108	*Taq*I and *Bam*HI	134 CL/P; 76 CPO	198 (birth registry)	Association between the *Bam*HI marker and bilateral CL/P; $p < 0.05$ (two-tailed exact test)	There is overlap of cases and controls in these two studies. Only sporadic cases were included, and the association found was between *TGFA* and the most severe cases.
	38	*Taq*I and *Bam*HI	196 CL/P; 114 CPO	198 (birth registry)	Association between the *Bam*HI marker and bilateral CL/P; $p < 0.05$ (two-tailed exact test)	
Japan	109	*Taq*I	71 CL/P; 14 CPO	117 (unspecified)	Association for CPO; $p < 0.05$ (two-tailed exact test)	There is almost complete overlap in the papers by Ozawa et al. (100) and Tamura et al. (109) and some overlap in the two papers by Machida et al. (111, 112). The paper by Tamura et al. (109) was used in the two meta-analyses (table 9).
	100	*Taq*I	71 CL/P; 13 CPO	117 (unspecified)	Association for CPO; $p < 0.04$ (two-tailed exact test)	
	101	*Taq*I	117 CL/P; 18 CPO	126 (unspecified)	No association	
	110	K, P, *Taq*I	56 CL/P	146 (hospital)	Association for the K marker; $p = 0.017$ (two-tailed exact test)	In the papers by Machida et al. (111, 112), the association grows stronger as the sample size grows. While some authors present data for the association with CPO, others present data for the association with CL/P.
	111	*Taq*I, 2A, H6	50 CL/P; 18 CPO	50 (unspecified)	No association	
	112	*Taq*I, 2A, H6	120 CL/P; 20 CPO	130 (unspecified)	Association for CL/P and the haplotype 2A-H6; $p = 0.0086$	

Table continues

TABLE 8. Continued

Location of study	Reference no.	*TGFA* genotype	Sample size and type		Reported results for association†	Highlights
			Cases	Controls (source)		
Philippines	113	*Taq*I, K	652 CL/P; 97 CPO	776 (hospital)	No association	
	114	Several	91 CL/P	96 (hospital)	Direct sequencing; no etiologic mutations found	
United States						
California	40	*Taq*I	447 CL/P; 215 CPO	734 (birth registry)	Association for mothers who smoked heavily; OR‡ = 6.1, 95% CI‡: 1.1, 36.6	Association was for both CL/P and CPO.
	90	*Taq*I	306 CL/P; 125 CPO	640 (birth registry)	Association for mothers who did not use multivitamins; OR = 3.0, 95% CI: 1.4, 6.6	Association was for both CL/P and CPO.
Iowa	15	*Taq*I, *Bam*HI, *Rsa*I	80 CL/P	100 (hospital)	Positive association; $p = 0.0047$ for *Taq*I and $p = 0.0052$ for *Bam*HI (two-tailed exact test)	In the paper by Ardinger et al. (15), 43% of cases had a positive family history of clefts.
	42	Two unspecified markers in the 3′-untranslated region of the gene	115 CL/P; 25 CPO	86 (hospital)	Association; $p = 0.04$ for CL/P and $p = 0.001$ for CPO	
	43	One unspecified marker in the 3′-untranslated region of the gene	20 CL/P cases for direct sequencing and an additional 120 CL/P cases for association studies	92 (hospital)	No association; no mutations found	
	22	*Taq*I, *Bam*HI, *Rsa*I, A, B, G, H, I, J, K, M, N, O, P, Q	52 CPO	192 (hospital)	Association; $p = 0.003$ for *Taq*I and $p = 0.017$ for K; no mutations found	
	34	*Taq*I, GGAA4D07	233 CL/P; 77 CPO	251 (hospital)	No association	
	44	*Taq*I	118 CL/P; 51 CPO	295 (hospital)	No association	
	26	Several	202 CL/P; 89 CPO	278 (hospital)	Five potential mutations in conserved regions identified in one CL/P case and four CPO cases	All potential mutations were in cases with cleft palate.
	114	Several	93 CL/P	96 (hospital)	Direct sequencing; no etiologic mutations found	
Maryland	39	*Taq*I	114 CL/P; 69 CPO	284 (birth registry)	Association between CPO and mothers who smoked; OR = 5.6, 95% CI: 1.36, 22.9	Association was seen only for CPO cases.
	91	*Taq*I	115 CL/P; 77 CPO	138 (birth registry)	No association	
	115	*Taq*I, D2S443	111 CL/P; 60 CPO	182 (birth registry)	No association	
Philadelphia, Pennsylvania	37	*Taq*I, *Rsa*I	100 CL/P	98 (unspecified)	Association for *Taq*I; $p = 0.03$ (two-tailed exact test)	
Vietnam	101	*Taq*I	130 CL/P; 77 CPO	15 (unspecified)	No association	

* Data reported in this table are for only nonsyndromic forms of clefts. All data relate to infant genotype.
† When a positive association is described for a specific phenotype, it implies that all other possible comparisons with other phenotypes were negative.
‡ CL/P, cleft lip with or without cleft palate; CPO, cleft palate only; OR, odds ratio; CI, confidence interval.

TABLE 9. Results from meta-analyses of the association between the transforming growth factor alpha (*TGFA*) *Taq*I marker and oral clefts*

Meta-analysis (reference no.)	Study location and population	Reference no.	Sample size (no.)			Odds ratio	95% confidence interval
			Cases	Controls	Total		
Mitchell (16)	United States, European descent	15	78	98		2.89	1.25, 7.10
	Australia, European descent	28	117	113		1.77	0.97, 3.32
	British	36	55	60		6.42	2.26, 22.25
	United States, European descent	37	83	84		2.07	1.02, 4.35
	French	38	98	99		0.70	0.27, 1.75
	United States, European descent	39	140	383		1.11	0.66, 1.84
	United States, European descent	40	190	379		1.05	0.65, 1.67
	All Europeans + European descent		761	1,216		1.59	1.28, 1.98
	United States, African-American	37	11	8		2.02	0.36, 14.37
	United States, African-American	39	22	69		1.05	0.10, 6.15
	United States, African-American	40	8	20		1.27	0.02, 25.95
	United States, Asian-American	37	6	6		1.00	0.06, 16.39
	Japanese	109	71	117		1.63	0.85, 3.09
	Filipino	102	NA†	NA		1.54	0.63, 4.32
	United States, Hispanic	40	85	175		1.28	0.45, 3.41
	Chilean	106‡	39	51		0.98	0.27, 3.38
Ioannidis et al. (17)	United States, European descent	15			352	2.9	1.4, 6.0
	British	36			230	6.0	1.2, 10.9
	Australia, European descent	28			460	1.9	1.0, 2.0
	French	38			394	0.75	0.29, 1.8
	United States—European descent, African-American, and Asian-American	37			394	2.0	1.2, 3.8
	United States—European descent and African-American	39			1,228	1.1	0.7, 1.9
	Japanese	109			376	1.8	0.9, 2.1
	Chilean	106			180	1.0	0.35, 2.0
	United States—European descent, African-American, and Hispanic	40			1,658	1.1	0.7, 1.8
	Total				5,272	1.7	1.2, 2.1

* Only data on cleft lip with or without cleft palate were considered from the studies included in these meta-analyses.
† NA, not available.
‡ Evidence of heterogeneity.

the 2p region (48, 51–53). One study of 10 families from Argentina, Mexico, and the United States did not show any linkage with the *TGFA* locus (54).

A targeted scan of candidate loci chosen on the basis of previous suggestive linkage and/or association with human families or suggestive animal-model data was carried out in three independent studies (47, 55, 56). Suggestive linkage and/or association between cleft lip/palate and the *TGFA* locus was found for Colombians ($p = 0.08$), Filipinos ($p = 0.01$), and North Americans from Ohio ($p = 0.005$) and Boston, Massachusetts/Texas ($p = 0.014$). This same approach was used to study candidate loci for cleft palate only in 24 Finnish families, but no linkage with the *TGFA* locus was found (57).

A meta-analysis of 13 genome scans (574 multiplex families, 3,584 genotyped individuals) of published and unpublished studies from Argentina, Australia, China, Colombia, England, India, Mexico, the Philippines, Syria, Turkey, and the United States (Iowa, Ohio, and Pennsylvania) showed suggestive linkage results (heterogeneity LOD score = 2.67; $p = 0.001$) for the *TGFA* locus on chromosome 2 (58).

Tooth agenesis

One study investigated the role of *TGFA* in tooth agenesis. In a Brazilian population, the affected-family-based controls and transmission disequilibrium tests showed an association between the *TGFA* C3827T marker and nonsyndromic tooth agenesis ($p = 0.01$ and $p = 0.02$, respectively). These results were confirmed by testing of the haplotype of *TGFA* *Taq*I-C3296T-C3827T by transmission disequilibrium test ($p = 0.02$). Interestingly, cases with at least one

incisor missing showed a borderline association with the *TGFA* markers C3296T ($p = 0.06$) and C3827T ($p = 0.05$), which supports the hypothesis that distinct types of teeth have independent genetic influences. No interactions with markers in the muscle segment homeobox 1 (*MSX1*) gene or the paired box 9 (*PAX9*) gene could be seen (59).

There is strong evidence supporting the possibility that cleft lip/palate and tooth agenesis could be related. In a Dutch family, an *MSX1* stop-mutation was associated with a concomitant cleft lip/palate and tooth agenesis phenotype (60). Syndromic forms of clefting, such as Van der Woude syndrome (caused by mutations in the interferon regulatory factor 6 (*IRF6*) gene) and autosomal-dominant Kallmann syndrome (caused by mutations in the fibroblast growth factor receptor 1 (*FGFR1*) gene), can present with oral clefts and tooth agenesis (61, 62). Patients with cleft lip/palate can have a frequency of tooth agenesis as much as six times higher than that of the general population (63, 64), and mice that are null for *Msx1* and *Pax9* have craniofacial anomalies that include cleft palate and tooth agenesis (65, 66). The association of *TGFA* with both cleft lip/palate and tooth agenesis is more evidence that these two defects share genetic predisposing factors.

Cancer

The role of the *TGFA Taq*I variant in cutaneous malignant melanoma (67, 68), breast cancer (69), and oral cancer (70) has been studied. The role of *TGFA* in human cancer is still unknown.

INTERACTIONS

For nonsyndromic oral clefts, gene-gene and gene-environment interactions have been suggested for *TGFA*.

An Australian study presented no evidence for an interaction between *TGFA* and the retinoic acid receptor variants (28). Retinoic acid, a naturally occurring form of vitamin A, is a recognized teratogen for cleft palate.

A genetic marker (*D2S378*) close to the *TGFA* gene showed LOD scores higher than 3.0 when Italian families linked to the 6p23 markers were analyzed (71). This result suggests not only a role for the *TGFA* locus in human clefting but also an interaction with a gene mapped at chromosome 6p23 in the development of the cleft.

A Norwegian study presented evidence of a strong effect of the *TGFA Taq*I rare allele among children homozygous for one common variant of the *MSX1* gene (72). That study did not suggest any possible interaction between *TGFA* and the transforming growth factor beta 3 (*TGFB3*) gene. However, a South American study did not provide evidence of an interaction between *TGFA* and *MSX1* (73).

A Brazilian study did not find evidence of an interaction between the rare *TGFA Taq*I allele and the 677T allele of the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene (74). However, a Norwegian study found a stronger effect of the homozygous form of the rare *TGFA Taq*I allele in children with one or two copies of the T allele at *MTHFR* C677T (relative risk = 4.0, 95 percent confidence interval (CI): 1.1, 13.9) than in children who were homozygous for the C allele (relative risk = 1.7, 95 percent CI: 0.2, 15.7) (75).

Environmental factors have been more extensively studied with regard to the association between *TGFA* genetic variants and oral clefts, and this research was recently reviewed (76). Among these environmental factors, maternal cigarette smoking during pregnancy presents the most compelling case for an interaction, because it has long been associated with a moderate increase in the risk of oral clefts (40, 77–84), though some studies have not confirmed such an association (39, 85–87). In a meta-analysis of the published literature (88), summary odds ratios associated with maternal smoking during pregnancy were 1.34 (95 percent CI: 1.25, 1.44) for cleft lip/palate and 1.22 (95 percent CI: 1.10, 1.35) for cleft palate only.

Evidence of interaction between *TGFA* marker alleles and maternal cigarette smoking during pregnancy in the risk of oral clefts can be seen in some studies, but not all (table 11). The biologic rationale for studying the interaction between *TGFA* and cigarette smoking is that bronchial epithelial cells, which respond to oxidants present in cigarette smoke by producing interleukin-8, make several ligands for the epidermal growth factor receptor, including TGFA (89).

Besides smoking, the use of vitamin supplements, ethanol, and recreational drugs and urinary tract infection have been evaluated (44, 75, 90, 91). Only periconceptional multivitamin use showed evidence for a *TGFA*-nutrient interaction in risk of clefting (75, 90). Compared with infants who were homozygous for the common *TGFA Taq*I genotype and whose mothers used multivitamins, increased clefting risks were observed for infants with the C2 genotype (homozygous and/or heterozygous) whose mothers did not use multivitamins. Risk estimates were 3.0 (95 percent CI: 1.4, 6.6) for infants with isolated cleft lip/palate in California and 4.5 (95 percent CI: 1.3, 15.7) for infants with isolated cleft palate only in Norway.

LABORATORY TESTS

There are no laboratory tests available as of yet, and laboratory testing is not indicated.

POPULATION TESTING

Molecular methods for determining the presence of the *TGFA* variants listed in table 1 have been published (22, 24, 26, 92, 93). All of the studies reviewed extracted genomic DNA from blood samples or blood-spot filter cards or used exfoliated oral cells. Genotyping methods used in the studies were consistent with the standard techniques of polymerase chain reaction (PCR), PCR-restriction fragment length polymorphism, kinetic PCR, and direct sequencing.

The *TGFA Taq*I, *Rsa*I, *Bam*HI, and *Hin*fI allelic variants can be determined by Southern blot or PCR-restriction fragment length polymorphism assay in agarose gels. The Southern blot allelic fragments will be detected using probes for the region of the gene indicated in primary references;

TABLE 10. Results from family-based studies of association/linkage between the transforming growth factor alpha (*TGFA*) gene and oral clefts*

Location of study	Reference no.	*TGFA* genotype	Study design	Sample size	Reported results for association/linkage†
Several‡	58	Several	Meta-analysis	568 CL/P§ multiplex families	Linkage; $p = 0.003$
Argentina	54	Unspecified	Genome-wide scan	2 CL/P multiplex families	No linkage
China	116	Unspecified marker with nine alleles	Family-based for linkage and TDT§	60 CL/P multiplex families	No association
	51	Several markers	Genome-wide scan (family-based for linkage and TDT)	36 CL/P multiplex families	No association and positive linkage (LOD§ score = 0.7) with the *TGFA* gene (71cM), but suggestive linkage was found for markers at 210 cM (LOD score = 1.45) and 227 cM (LOD score = 1.91).
Colombia	47	D2S443	Family-based for linkage and TDT	35 CL/P multiplex families	Suggestive linkage; $p = 0.077$ (nonparametric test)
	58	D2S1364-D2S1777	Family-based for linkage and TDT	49 CL/P multiplex families	Suggestive linkage; D2S443, LOD score = 0.68; D2S1394, $p = 0.08$ (nonparametric tests)
South America¶ (ECLAMC§)	35	*Taq*I	Family-based for TDT	199 CL/P mother–affected-child pairs; 24 CPO§ mother–affected-child pairs	Not conclusive (marker was not informative)
South America (ECLAMC)	73	C3827T#	Family-based for TDT	199 CL/P mother–affected-child pairs; 24 CPO mother–affected-child pairs	Borderline association for CPO; $p = 0.088$ (likelihood ratio statistic)
England	117	*Taq*I, *Bam*HI, *Rsa*I	Family-based for linkage	8 CL/P multiplex families	No linkage
	46	*Taq*I	Family-based for TDT	71 CL/P trios (mother-father-affected child) (cases were also ascertained in the United States)	Association; $p < 0.005$
	118	*Taq*I	Family-based for TDT	130 CL/P trios (mother-father-affected child)	Borderline association; $p = 0.062$
	50	Several between markers D2S2368 and D2S1790	Genome-wide scan (family-based for linkage)	92 CL/P sibling families	Linkage; $p = 0.04$ (nonparametric test)
Finland	57	21 unspecified markers	Family-based for linkage	24 CPO multiplex families	No linkage
India	119	K	Family-based for linkage	14 CL/P multiplex families	No linkage. Higher frequency of primer K allele 3 variant was seen among families with cleft lip with cleft palate, while families with cleft lip only showed a higher frequency of the primer K allele 2 variant.
	53	37 markers	Family-based for linkage	38 CL/P multiplex families	No linkage
Italy	120	*Taq*I	Family-based for linkage and TDT	40 CL/P multiplex families	No association
	71	*Taq*I and other markers	Family-based for linkage	38 CL/P multiplex families	Linkage for families linked to chromosome 6p23 (marker D2S378 with LOD scores from 3.52 to 3.96)
Mexico	121	D2S443	Family-based for linkage	22 CL/P multiplex families	No linkage
	54	Unspecified	Genome-wide scan	6 CL/P multiplex families	No linkage

Table continues

they are: *Taq*I, 3.0 kilobases (common allele) and 2.7 kilobases (rare allele); *Rsa*I, 1.5 kilobases (common allele) and 1.2 kilobases (rare allele); *Bam*HI, 7.0 kilobases (common allele) and 4.0 kilobases (rare allele); and *Hin*fI, 2.9 kilobases (common allele) and 2.5 kilobases (rare allele). For the *TGFA Taq*I variant, a PCR assay with allelic fragments of 117 base pairs (common allele C1) and 113 base pairs (rare allele C2) is available (92).

The P primer variant alleles have been detected by single-strand conformation polymorphism. The products are

TABLE 10. Continued

Location of study	Reference no.	*TGFA* genotype	Study design	Sample size	Reported results for association/linkage†
Norway	72	*Taq*I	Family-based for TDT	157 CL/P and 63 CPO trios (mother-father-affected child)	Threefold risk among CPO children who were homozygous for the rare *Taq*I allele
Philippines	122	Unspecified	Family-based for linkage and TDT	30 CL/P multiplex families	No linkage
	55	C3827T	Family-based for linkage and TDT	36 CL/P multiplex families and an additional 70 families for replication	Linkage; $p = 0.01$ (nonparametric test)
Sweden	123	D2S123, D2S337, D2S378, D2S380	Family-based for linkage	19 CL/P multiplex families	No linkage
Syria	52	Unspecified	Family-based for linkage	2 CL/P multiplex families	Linkage to D2S1356, located at chromosome 2p16.3; LOD score = 1.6, $p < 0.01$
Turkey	48	Unspecified	Family-based for linkage and TDT	18 consanguineous CL/P families	Linkage for D2S1777 (near *TGFA*), LOD score = 1.45; association between CL/P and *TGFA*, $p = 0.053$
United States					
Boston, Massachusetts/Texas	56	D2S2368, D2S86, D2S1790, D2S1387	Family-based for linkage and TDT	14 CL/P multiplex families	Association between CL/P and the markers D2S2368 ($p = 0.014$), D2S1387 ($p = 0.0025$), and D2S338 ($p = 0.028$)
Iowa	34	*Taq*I, GGAA4D07	Case-control and family-based for TDT	233 CL/P cases, 77 CPO cases, and 251 hospital controls	No association
	54	Unspecified	Genome-wide scan	2 CL/P multiplex families	No linkage
Maryland/Washington, DC	121	D2S443	Family-based for linkage	35 CL/P multiplex families	No linkage
	103	D2S443	Family-based for TDT	110 CL/P trios (mother-father-affected child); 50 CPO trios (mother-father-affected child)	Association; $p = 0.03$ (likelihood ratio statistic)
	124	D2S443	Family-based for TDT	186 CL/P trios (mother-father-affected child); 83 CPO trios (mother-father-affected child)	No association
Minneapolis, Minnesota/Kansas/Texas	125	*Taq*I, *Bam*HI, *Rsa*I	Family-based for linkage	12 CL/P multiplex families	No linkage
Ohio	47	D2S443	Family-based for linkage and TDT	12 CL/P multiplex families	Suggestive linkage; $p = 0.077$ (nonparametric test)
	58	D2S1364-D2S1777	Family-based for linkage and TDT	13 CL/P multiplex families	Linkage; D2S1342, $p = 0.005$, LOD score = 0.65 (nonparametric tests)
Pennsylvania/Texas	46	*Taq*I	Family-based for TDT	71 CL/P trios (mother-father-affected child) (cases were also ascertained in England)	Association; $p < 0.005$

* Data reported in this table are for only nonsyndromic forms of clefts.

† When a positive association/linkage is described for a specific phenotype, it implies that all other possible comparisons with other phenotypes were negative.

‡ Populations included in this meta-analysis were from Argentina, Australia, China, Colombia, England, India, Mexico, the Philippines, Syria, Turkey, and the United States (Iowa, Ohio, and Pennsylvania).

§ CL/P, cleft lip with or without cleft palate; TDT, transmission disequilibrium test (see Spielman et al. (49)); LOD, logarithm of the odds; ECLAMC, Estudio Colaborativo Latino Americano de Malformaciones Congénitas; CPO, cleft palate only.

¶ This study comprised information from hospitals in Argentina, Bolivia, Brazil, Chile, Ecuador, Paraguay, Uruguay, and Venezuela.

C3827T is a C-to-T substitution at nucleotide 3827.

fragments 369 (common allele) and 365 (rare allele) base pairs long (22).

The K primer allelic variants are determined using a combination of single-strand conformation polymorphism and denaturing gradient gel electrophoresis. The primers for this four-allele polymorphism amplify a 345-base-pair fragment. In a single-strand conformation polymorphism gel, allele 3 is the fastest-migrating band, and alleles 2 and 4

TABLE 11. Results from studies of the interaction between transforming growth factor alpha (*TGFA*) genetic variants and cigarette smoking

Location of study	Reference no.	*TGFA* genotype	Reported results
Denmark	41	*Taq*I	No evidence of interaction
South America* (ECLAMC†)	35	*Taq*I	Not conclusive; marker not informative
England	118	*Taq*I	No evidence of interaction
Norway	75	*Taq*I	No evidence of interaction
United States			
California	40	*Taq*I	Risk for clefting when child had the rare *TGFA Taq*I allele and the mother smoked 20 or more cigarettes/day; for CL/P†, OR† = 2.3, 95% CI†: 1.1, 5.1; for CPO†, OR = 2.8, 95% CI: 1.1, 7.2
Iowa	44	*Taq*I	No evidence of interaction
Maryland	39	*Taq*I	CPO infants carrying the rarer *TGFA Taq*I allele who were exposed to maternal smoking of 10 or fewer cigarettes/day had a 6.16-fold increased risk (95% CI: 1.09, 34.7), while similar infants whose mothers smoked more than 10 cigarettes/day had an 8.69-fold increased risk (95% CI: 1.57, 47.8).
	91	*Taq*I	Transmission disequilibrium test showed significant interaction between maternal smoking and the transmission of allele markers near *TGFA*
	103	D2S443	No evidence of interaction
	115	*Taq*I, D2S443	No evidence of interaction
	124	D2S443	No evidence of interaction
Meta-analysis	126	*Taq*I	CPO infants carrying the rarer *TGFA Taq*I allele who were exposed to maternal smoking had a 1.95-fold increased risk (95% CI: 1.22, 3.10). *TGFA* genotype did not increase risk of CL/P, regardless of maternal smoking status.

* This study comprised information obtained from hospitals in Argentina, Bolivia, Brazil, Chile, Ecuador, Paraguay, Uruguay, and Venezuela.
† ECLAMC, Estudio Colaborativo Latino Americano de Malformaciones Congénitas; CL/P, cleft lip with or without cleft palate; OR, odds ratio; CI, confidence interval; CPO, cleft palate only.

comigrate. In a denaturing gradient gel electrophoresis analysis, alleles 1 and 4 comigrate. By performing both experiments, it is possible to distinguish the four alleles, especially if positive controls with known genotypes are included (22).

For the variants C3296T and C3827T, kinetic PCR- or direct-sequencing-based assays have been described. All other variants described in table 1 were originally detected by direct sequencing. Older techniques (single-strand conformation polymorphism, denaturing gradient gel electrophoresis, or Southern blot) could probably be replaced by newer genotyping methods using available sequence data.

OTHER POTENTIAL PUBLIC HEALTH APPLICATIONS

Other potential public health applications are dependent on confirmation that particular mutations or variants increase the risk of oral clefts or cancer.

CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

Genetic epidemiologic data support the hypothesis of a small effect of *TGFA* on clefting in humans. The attributable risk of *TGFA* for clefts was calculated to be between 1.21 and 1.23, or a 20 percent increase in risk to offspring and siblings attributed to *TGFA* (94).

The magnitude of the association between *TGFA* and oral clefts in persons of European descent is 0.62; that is, the frequency with which the ''rare'' *TGFA* marker allele is transmitted from heterozygous parents to affected offspring is 62 percent instead of the expected 50 percent (95). This statistic further demonstrates the effect of *TGFA* on oral clefts in humans.

While no missense, stop-codon, or splice variants were detected that could provide direct evidence of *TGFA* protein dysfunction in clefting, five mutations in 3′-untranslated conserved regions, which could play a role in message stability or tissue-specific targeting, were described (26). These mutations were not found in controls. In aggregate, these mutations showed a marginal association, suggesting that, as a group, such mutations may be responsible for clefting. Although this is only weakly supportive evidence—since the statistical evidence as a whole continues to support a role of *TGFA* in clefting and since the exact consequences of mutations in the 3′-untranslated region are not yet fully understood—*TGFA* remains on any list of candidate genes for clefting.

The *Tgfa* knockout mice demonstrated no cleft phenotype (13, 14), suggesting that *Tgfa* may act as a modifier gene rather than being a necessary and sufficient determinant (96, 97). There is evidence supporting this in the studies presented in tables 8–10 (see ''Highlights'' column in table 8

and ''Reported results'' column for Asian Indians and Italians in table 10). One hypothesis is that the *TGFA* locus modifies the expression (severity) of the cleft lip/palate trait. However, it is not clear what aspect of expression (presence or absence of palate fusion) is influenced by the *TGFA* locus.

In summary, the role of *TGFA* in clefting appears small but significant, and mutations in this gene may represent a rare cause of clefting in humans. The conflicting results seen in the literature are partially caused by differences in both study design and populations. *TGFA* is probably a genetic modifier of clefting in humans, which is concordant with the oligogenic model suggested for nonsyndromic oral clefts.

Investigators in future studies should focus on understanding the possible role of common polymorphic variants in the development of oral clefts. The possible interaction between *TGFA* and other clefting-related genes, such as *MSX1*, must also be explored. A more complete clinical description of affected persons, including the severity and laterality of clefts and the presence of hypodontia and other dental anomalies, might be useful in future studies. To address these issues, investigators will need to use study designs that remove bias due to differences in family history, clinical description (cleft type, severity, laterality, association with other oral and craniofacial anomalies), genetic markers, and ethnic background (to clarify possible differences in association patterns for distinct population groups).

INTERNET SITES

A list of useful Internet sites is provided in the Appendix.

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APPENDIX

Internet Sites

Atlas of Genetics and Cytogenetics in Oncology and Haematology: http://www.infobiogen.fr/services/chromcancer/Genes_gc/GC_TGFA.html

Biology of the Mammary Gland: http://mammary.nih.gov/

Cancer Genetics Web: http://www.cancerindex.org/geneweb/

Gene Cards: http://biostatpub2.mdanderson.org/genecards/index.shtml

Human Protein Reference Database: http://www.hprd.org/protein/07522

Information Hyperlinked Over Proteins: http://www.ihop-net.org/UniPub/iHOP/gs/125537.html

Murray Laboratory genetics information server, University of Iowa: http://genetics.uiowa.edu/data/candidateGenes/TGFA.html

Mutation Database: http://mutdb.org

National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov

University of California, Santa Cruz Genome Bioinformatics browser, version 24: http://www.genome.ucsc.edu

Utah Genome Depot: http://www.genome.utah.edu